

Research Article

ESTIMATION AND COMPARISON OF CLINICAL STAGING AND CANDIDAL COLONY COUNTS IN ORAL LEUKOPLAKIA BEFORE AND AFTER TREATMENT WITH 1 % TOPICAL ANTIFUNGAL THERAPY

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ABSTRACT

Aim: The aim of this study to observe and evaluate effectiveness of 1 % Topical clotrimazole therapy on Clinical and Histopathologic behavior of Oral Leukoplakia and to compare the Candida colony count in saliva before and after 1% Topical clotrimazole therapy in patients with Oral Leukoplakia.

Methods: Clinical behavior of 50 cases of oral leukoplakia were assessed according to the OLEP staging. Samples of unstimulated saliva were collected and candidal colonies counted. Patients were prescribed 1% topical Clotrimazole antifungal therapy thrice daily for 15 days. Same procedures were repeated after treatment to compare pre and post OLEP staging of Leukoplakia and colony forming units.

Results: Clinical and histopathological staging of leukoplakia showed statistically significant difference in pre and post treatment with 1% topical Clotrimazole antifungal therapy, as well as in salivary candida colony count. Paired t test showed statistically significant reduction in CFU with a p value of <0.001

Conclusion: Candidal infection may be one of the most important factors inducing dysplasia of epithelia and malignant transformation of oral leukoplakia. Since the lesion showed significant improvement in the clinical staging of leukoplakia as well as decrease in the candidal colony count in saliva following antifungal therapy, 1% Topical clotrimazole is recommended for oral leukoplakia patients to eliminate the candidal infection and further improvement in the clinical staging and prevention of progression of leukoplakia. Hence, prescription of 1% topical clotrimazole antifungal therapy can be recommended to all oral leukoplakia patients as a part of regular management protocol for oral leukoplakia.

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INTRODUCTION

Oral Leukoplakia is the most common potentially malignant disorder of the oral mucosa. It is defined as “a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion”.^[1] Two clinical types of Leukoplakia are being recognized, the homogeneous and the non-homogeneous type.^[2] The annual rate of malignant transformation of Oral Leukoplakia has been reported to be 1.36%.^[3] Increased malignant potential may be associated with certain clinical characteristics such as lesion type, size, site, dysplasia, and tobacco use. Generally non-homogeneous

leukoplakia carries a greater risk of malignant transformation than the homogeneous type which is related to the presence of epithelial dysplasia.^[4]

Candidiasis is one of the common mycotic infections of the oral cavity which manifest in various forms. The association of oral leukoplakia with candida was first reported by Cornea *et al.* and Jepsen and Winther in 1965.^[5] Candida invasion has been suggested to be a significant risk factor for malignant transformation of oral Leukoplakia.^[3] Though the role of Candida on epithelial dysplasia is not fully understood, antifungal therapy is recommended to be a routine treatment for patients with homogenous and non homogenous Leukoplakia.^[6] An observation period of 2–4 weeks interval is recommended for possible regression or disappearance of a

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white lesion after elimination of possible causative factors before taking a biopsy.^[7] Since there were no studies in the literature correlating the staging of Leukoplakia and candida colonization in the oral cavity before and after treatment with topical antifungal therapy, the present study was undertaken to observe and compare the change in clinical staging of oral leukoplakia and estimate the candida colony formation from the saliva before and after treatment with topical antifungal agent, 1 % clotrimazole.

MATERIALS AND METHOD

This was an in vivo observational study conducted in a dental hospital after obtaining clearance from institutional review board and ethical committee. 50 patients with clinical diagnosis of oral leukoplakia were included in the study. Patients with chronic systemic illness, oral cancer, medically compromised patients, those on corticosteroid therapy, immunosuppressive drugs, allergic to clotrimazole were excluded from the study.

A case selection criterion was based on OLEP (2000) Leukoplakia staging system proposed by van der Waal & Axéll *et al* (Table 1).

Table 1 Classification and Staging System for Oral Leukoplakia (Olep) -Van Der Waal (2002)

L (size of the leukoplakia)	Size of single or multiple leukoplakias together
L ₁	≤2 cm
L ₂	Size of single or multiple leukoplakias together 2-4 cm
L ₃	Size of single or multiple leukoplakias together ≥4 cm
L _x	Size not specified
P (pathology)	No epithelial dysplasia (includes "no or perhaps mild epithelial dysplasia")
P ₀	Distinct epithelial dysplasia (includes "mild to moderate" and "moderate to possibly severe" epithelial dysplasia)
P _x	Absence or presence of epithelial dysplasia not specified in the pathology report
OLEP staging system:	
Stage I	L1P0
Stage II	L2P0
Stage III	L3P0 or L1L2P1
Stage IV	L3P1

After the assessment of clinical staging, subjects were asked to rinse their mouth with 10 ml of distilled water for 2 mins and collect it into a sterile container following the oral rinse technique described by Samaranayake *et al.* (1994). The samples were inoculated onto Sabouraud dextrose agar media and incubated at 37°C for 48 h. Microscopic morphology (Gram stain) and germ tube test were carried out to differentiate *C. albicans* from other species. Candida colony forming units (CFU) were counted using colony counter. Candida isolates recovered by culture following were inoculated on the chromogenic culture medium CHROM agar Candida™. This medium permits the presumptive identification of several clinically important Candida species including *C. albicans* based on colony color.

Patients were counseled for habit cessation and prescribed topical antifungal agent, 1% Clotrimazole and advised to apply on the lesion thrice daily for 15 days. Method of application was explained and Medication compliance chart was given to assess adherence to the regimen. Patients were

recalled after 2 weeks and assessed for clinical staging based on size, excluding the pathology (c-OLEP) and saliva collected to estimate the candida colony count. The obtained data was tabulated and subjected to statistical analysis. Statistical analysis was done using SPSS software version 20. Paired t test was done to compare the changes before and after treatment with 1% topical Clotrimazole with p value of 0.001.

RESULTS

There was no drop out and all the patients followed the instructions given. The age distribution ranged from 20-70 years, with a mean age of 50.14 ± 10.76 years. Our study showed a peak occurrence of Leukoplakia between the age groups of 51-60 years. (Table 2)

Table 2 Age Distribution of Leukoplakia Patients

Age	No of patients	Percentage
21-30	3	6
31-40	5	10
41-50	12	24
51-60	18	36
61-70	12	24
Total	50	100

Out of 50 patients, 47 cases (94%) were males and 3 cases (6%) were females. The type of lesions were 39 cases (78%) of homogenous leukoplakia and 11cases (22%) of non homogenous leukoplakia.

The study group showed different sites of involvement in the oral cavity. Among which, 45 cases (90%) involved buccal mucosa, 2 cases (4%) involved labial mucosa and vestibular region and 1 case (2%) in retro molar area.

Pretreatment staging and candida colony count

OLEP staging: The pre treatment staging including the size of the lesion (L) and histopathology (P) of Leukoplakia showed 20 cases (40%) in grade 1; 19 cases (38%) in grade 2; 5cases (10%) in grade 3 and 6 cases (12%) in grade 4

Histopathological grading of the lesion showed 20 cases (40%) with mild dysplasia, 1 case (2%) with moderate dysplasia and 29 cases (58%) without any dysplastic features.

CFU: The candidal count was in the range of 800-8000 CFU/ml before treatment. 2% of cases showed colony count in the range of 0-600 CFU/ml, 33% of cases showed colony counts in the range of 800-2000 CFU/ml, 6% of cases showed colony counts in the range of 2000-4000 CFU/ml; 6% cases showed colony counts in the range of 4000-6000 CFU/ml and 8% cases in the range of 6000-8000 CFU/ml.

Post treatment staging and Candida colony count

c-OLEP: The post treatment clinical staging of Leukoplakia based on the size of the lesion (L) and clinical sub classification (C) *ie*, Homogenous (C1) or non homogenous (C2), showed 43 cases (86%) in grade 1; 3 cases (6%) in grade 2 and no cases were observed in grade 3 and 4 cases (8%) in grade 4. (Table 3)

CFU: Following treatment, 70 % of the cases showed complete reduction /absence of colony formation. 14% cases showed reduction to the range of 800-2000, 12 % cases showed reduction to the range of 2000-4000 and in 4% cases got reduced to 4000-6000 from a higher colony count.

Table 3 Clinical Staging of Leukoplakia

Staging Before Treatment(Olep)	No Of Cases	Percentage Before Treatment	Staging After Treatment (C)	No Of Cases	Percentage after treatment
L1P0	20	40.0	L1C1	20	40.0
L2P0	8	16.0	L2C1	0	0
L3P0 or L1L2P1	16	32.0	L1C1	19	38.0
			L3C1	0	0
			L2C1	1	2.0
			L1C1	4	8.0
L3P1	6	12.0	L3C2	4	8.0
			L3C1	0	0
			L2C1	2	4.0
			L1C1	0	0

L1 Size of single or multiple leukoplakias together ≤ 2 cm
 L2 Size of single or multiple leukoplakias together 2+4 cm
 L3 Size of single or multiple leukoplakias together ≥4 cm
 P (pathology)
 P0 No epithelial dysplasia
 P1 Distinct epithelial dysplasia (includes "mild to moderate" and "moderate to possibly severe" epithelial dysplasia; equals OIN grades 1 and 2)
 C1=homogeneous; C2=non-homogeneous

Mean candidal CFU before treatment was 2568±2388 and mean CFU following treatment was 888±1571. Paired t test was done which showed statistically significant reduction in CFU with a p value of <0.001 (Table 4)

Table 4 Comparison of Range And Percentage of Candida Colony Counts Before And After Treatment

Candida Colony count	No of cases in pre treatment (%)	No of cases in post treatment (%)
0-600	2.0	70.0
800-2000	66.0	14.0
2000-4000	12.0	12.0
4000 -6000	12.0	4.0
6000 -8000	8.0	0

DISCUSSION

Oral leukoplakia is regarded to be a premalignant or potentially malignant or precancerous lesion. The annual rate of malignant transformation of 1.36% (0.69%-2.03%) has been reported based on the various populations and geographical areas.^[3]

Candida albicans is the most prevalent fungal species associated with oral leukoplakia.^[8] Candida albicans and several related candida species are opportunistic pathogens which live as benign commensals in the oral cavities of healthy individuals. Patients with candidiasis have >400 CFU per ml of saliva. Leukoplakia harboring candidal hyphae constitutes 7-50% of all leukoplakias.^[5] Candida invasion has been suggested to be a significant risk factor of malignant transformation of oral leukoplakia.^[9]

A staging system for oral Leukoplakia combining the clinical aspects and histopathologic findings (OLEP) has been proposed which helps to have a better knowledge and understanding about oral leukoplakia. If the staging system should be used for studies in which no biopsies are available, e.g. in epidemiological surveys, (in our study, follow up biopsy was not recommended due to ethical issues) one could use a symbol for a clinical (C) subdivision of OLEP (C1=homogeneous; C2=non-homogeneous) instead of the one for the pathology. With reference to the original classification and staging system (van der Waal, 2000), this would result in a c-OLEP system, recognizing four stages. (Table 5)

Table 5 A Modified Classification And Staging System For Oral Leukoplakia (C-Olep) -Van Der Waal (2000)

Stage I	L1C1,
Stage II	L2C1,
Stage III	L3C1 or L1L2C2
Stage IV	L3C2.

C1-Homogenous
 C2-Non Homogenous

Among the types of Leukoplakia, the homogenous type is the more prevalent than Non-homogenous.^[10] Our study also showed similar finding, Homogenous Leukoplakia 39 (78%) was more prevalent than the non-homogenous leukoplakia 11 (22%). However the percentage of cases of Homogenous Leukoplakia in our study was higher when compared to other studies. The reasons for the higher incidence of homogenous leukoplakia in the present study are multifactorial. It could be due to the variations in the type of tobacco product, duration and frequency of tobacco product consumed; with or without consumption of slaked lime, combined with alcohol usage prevailed among the South Indian population.^[11]

Most interesting observation was the significant reduction in the size of the lesion and improvement in the Clinical Stages of Leukoplakia, Pre and Post cotrimazole topical antifungal therapy. (Figure 1,2)

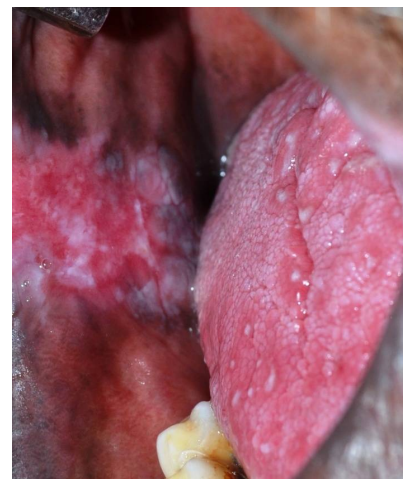


Fig 1 Pre treatment clinical photograph showing non homogenous oral leukoplakia



Fig 2 Post treatment clinical photograph showing homogenous oral leukoplakia

All the 20 cases with L1P0 showed no difference in staging, whereas 8 cases in L2P0 stage turned to L1P0 after treatment and 16 cases in L3P0 stage before turned into L1P0 in 15 cases and L2P0 in 1 case; 6 cases in L3P1 turned to L2P0 and L1P0 in 5 cases and 1 case respectively. Results obtained were statistically significant with a p value of < 0.001 which suggests the significant role of candida in the progressive stages of leukoplakia and the role of topical antifungal therapy in the regression of stages Oral Leukoplakia.

Assimilating the observed results, 48% of cases showed improvement in staging *ie.* 24 cases got reverted to L1P0 stage; 6(12%) cases got reverted to L2P0 from a higher stage and 20(40%) cases remained in L1P0 stage following treatment.

An Important observation was the post treatment reduction in the size of the clinical lesion among all the 20 cases in L1P0 stage. This regression of the lesion couldn't be documented in the form of staging because of the inherent nature of the staging system where a lesion $< 2\text{cm}$ however small, has to be graded as L1. Hence a pretreatment lesion which is 2cm in size regressing to 0.5 cm post treatment still is graded as L1

The present study showed 11 cases of non homogenous leukoplakia, in which 7 cases turned into homogenous leukoplakia post treatment with application of 1% w/v clotrimazole antifungal therapy. There is a significant change in clinical staging of leukoplakia cases which strongly signifies the role of Candida in Leukoplakia.

Another important observation in our study is the significant reduction in candida colony counts following the application of 1% w/v clotrimazole. (Figure 3, 4) The candidal count in the range of 800-8000 CFU/ml before treatment showed complete absence as well as reduction following treatment with antifungal treatment. Mean candidal CFU before treatment was 2568 ± 2388 and following treatment was 888 ± 1571 . Paired t test showed statistically significant reduction in CFU with a p value of < 0.001 . A study

conducted by Cao (2007) on 100 cases of healthy control group, 110 cases of oral leukoplakia and found significant correlation within saliva culture results and he concluded that Candidal infection may be one of the most important factors inducing dysplasia of epithelia and malignant transformation of oral leukoplakia.^[12] The reduction in the candida colony count before and after application of antifungal therapy can be compared with study results of pragati (2013).^[13] So saliva culture should be taken as routine for patients with oral leukoplakia.



Fig 3 Pre treatment candida colony photograph



Fig 4 Post treatment candida colony photograph

CONCLUSION

There are conflicting evidences regarding the presence of *C. albicans* with malignant and premalignant lesions. Though the association of *C. albicans* in the malignant transformation of potentially malignant lesions is not proven with strong evidences, the present study assessing the clinical staging of oral leukoplakia and salivary candidal colony count before and after treatment with antifungal therapy showed improvement in the clinical staging as well as reduction in the candida colony. High candida colony counts in saliva before treatment suggests the possible correlation of candida with the clinical stage of leukoplakia. It is evident from the significant reduction of the candidal colony count and improvement in the clinical staging after the application of 1% w/v clotrimazole, the possible role of candida in progression of leukoplakia.

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